Acute Injury with Intravenous Iron and Concerns Regarding Long-Term Safety

Kalkidan Bishu and Rajiv Agarwal

Division of Nephrology, Department of Medicine, Indiana University School of Medicine, and Richard L. Roudebush VA Medical Center, Indianapolis, Indiana

Intravenous iron is widely used to maintain adequate iron stores and prevent iron deficiency anemia in patients with chronic kidney disease, yet concerns remain about its long-term safety with respect to oxidative stress, kidney injury, and accelerated atherosclerosis, which are the subjects of this review. Three parenteral iron formulations are available for use in the United States: Iron dextran, iron gluconate, and iron sucrose. Iron dextran, especially the high molecular form, has been linked with anaphylactoid and anaphylactic reactions, and its use has been declining. A portion of intravenous iron preparations is redox-active, labile iron available for direct donation to transferrin. In vitro tests show that commonly available intravenous iron formulations have differing capacities to saturate transferrin directly: Iron gluconate > iron sucrose > iron dextran. Intravenous iron treatment produces oxidative stress, as demonstrated by increases in plasma levels of lipid peroxidation products (malondialdehyde), at a point that is much earlier than the time to peak concentration of catalytically active iron, suggesting a direct effect of iron sucrose on oxidative stress. Furthermore, iron sucrose infusion produces endothelial dysfunction that seems to peak earlier than the serum level of free iron. Intravenous iron sucrose infusion also has been shown to produce acute renal injury and inflammation as demonstrated by increased urinary albumin, enzyme (N-acetyl-β-glucosaminidase), and cytokine (chemokine monocyte chemoattractant protein-1) excretions. Although the long-term dangers of intravenous iron are unproved, these data call for examination of effects of intravenous iron on the potential for long-term harm in patients with chronic kidney disease.

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Address correspondence to: Dr. Rajiv Agarwal, Department of Medicine, VAMC, 111N, 1481 West 10th Street, Indianapolis, IN 46202. Phone: 317-554-0000 ext. 2241; Fax: 317-554-0298; E-mail: ragarwal@iupui.edu

Intravenous Iron Formulations Differ in Their Ability to Donate Iron to Transferrin

Oxidative stress, that is iron catalyzed, is more likely to occur when iron is not bound to transferrin and is catalytically active.

To determine whether intravenous iron donates iron to transferrin directly and whether any differences exist between dextran and nondextran iron preparations Agarwal (12) performed an in vitro experiment. Transferrin was incubated with various formulations of intravenous iron in the same concentration for various lengths of time. Figure 1 demonstrates different degrees of transferrin saturation (TSAT) by urea-PAGE after incubation of transferrin with iron gluconate and iron sucrose. Transferrin has two binding sites, and when bound to iron, apotransferrin is converted to monoferric or diferric transferrin. Urea-PAGE analysis is able to separate apotransferrin, monoferric, and diferric transferrin. Iron dextran after 3 h of incubation with transferrin showed no direct transfer of iron from iron dextran to transferrin. Conversely there was substantial transfer of iron to transferrin for both iron sucrose and iron gluconate in a time-dependent and dose-dependent manner (Figure 2). The nondextran formulations also differ significantly in their ability to donate iron; iron gluconate saturates transferrin faster than iron sucrose. Other investigators have made similar observations (13,14). The risk-benefit ratio of the various iron preparations, however, cannot be ascertained by the rate of iron transfer because the determinations described were made using in vitro studies and such analysis would require clinical trials. Nevertheless, these data suggest a hypothesis that commercially available intravenous iron formulations may have clinical differences in their potential to cause oxidative stress and injury and repair anemia.
Iron Generates Oxidative Stress in Humans

Patients who are on dialysis are at a higher risk for cardiovascular disease (CVD) than is the general population. There is a consensus that atherosclerosis, an important cause of cardiovascular mortality in patients with ESRD (15), is a state of heightened oxidative stress (16). Likewise, CKD is a highly pro-oxidant state (17,18).

Several studies have examined the effects of intravenous iron therapy on oxidative stress. Zager et al. (19) studied the safety of various intravenous formulations of iron in terms of lipid peroxidation and cell injury in in vitro systems using cell cultures or human proximal tubular cells, endothelial cells, and tubules. They showed that when cell viability is assessed in vitro after exposure to iron sucrose, iron gluconate, and iron dextran preparations, iron sucrose is the most toxic and iron dextran the least. They also showed that all three agents resulted in a massive and similar increase in lipid peroxidation. Whole-animal studies show that intravenous administration of iron dextran in rats with chronic renal failure also induces oxidative stress (20,21).

Consistent with the findings in animals, an exacerbation of oxidative stress occurs after intravenous infusion of iron sucrose in dialysis patients, as demonstrated by an increase in plasma concentrations of malondialdehyde (MDA), one of the end products of the peroxidation of polyunsaturated fatty acids (22). Roob et al. (23) demonstrated that intravenous iron leads to the generation of MDA, with plasma concentrations reaching a peak at 30 min after the start of an infusion of 100 mg of intravenous iron sucrose in patients who are on hemodialysis. When vitamin E was given along with intravenous iron, there was some mitigation of oxidative stress, although MDA levels still were higher than for patients who did not receive intravenous iron. They also measured bleomycin-detectable iron, a marker for redox-active iron, and found a rapid increase in plasma bleomycin-detectable iron. In fact, Tovbin et al. (24) found that intravenous iron leads to greater protein oxidation in hemodialysis patients when they have a greater amount of inflammation. These data support the hypothesis that the administration of intravenous iron generates redox-active free iron and oxidative stress.

Iron Generates Oxidative Stress in Humans

Intravenous Iron, Atherosclerosis, and Endothelial Dysfunction

CVD is the primary cause of death in patients with ESRD (15), and atherosclerosis is the major cause of ischemic heart disease in dialysis patients. Studies have found that common carotid artery intima-media thickness, an early marker of atherosclerosis, is associated with annual intravenous iron dose (25,26), serum ferritin (25), and markers of oxidant-mediated protein damage (25) in patients with ESRD. The current paradigm for the pathogenesis of atherosclerosis emphasizes the central role of endothelial dysfunction (27) that seems to be mediated through oxidative stress (28). Endothelial dysfunction and injury trigger an inflammatory cascade that, if unabated, could lead to remodeling of the arterial wall. That iron may be important in mediating atherosclerosis is supported by a recent study from the Netherlands that demonstrated increased adhesion of normal human monocyte cells to human umbilical
endothelial cells that were treated with the serum of patients with hemochromatosis (29). The expression of the adhesion molecules intercellular adhesion molecule-1, vascular cellular adhesion molecule-1, and E-selectin also was positively correlated to free iron levels.

A few studies demonstrated modulation of endothelial function by intravenous iron administration. Rooyakkers et al. (30) administered intravenous iron sucrose to people with normal kidney function and evaluated endothelial function, free iron levels, and free-radical activity (Figure 3). Intravenous iron sucrose increased free radical activity and reduced flow-mediated dilation. Intravenous iron did not impair nitroglycerin-induced dilation, which is a measure of endothelium-independent vessel function. At 10 min after the administration of iron sucrose, there was a substantial increase in free iron that continued to rise up to 4 h after infusion. If free iron is considered to be directly responsible for the endothelial dysfunction, then it would be expected that at 4 h, there would be greater endothelial dysfunction than at 10 min. Instead, endothelial dysfunction was maximal at 10 min and had normalized by 4 h, suggesting that free iron is not the direct cause for the dysfunction. Oxidative stress, maximally generated at 10 min, was better correlated with impairment of endothelial function.

A study by Zheng et al. (31) evaluated in a double-blind, controlled experiment the effect of iron sucrose given in a recommended dose (100 mg) but at a slower rate (30 min rather than 5 min) to assess its effect on endothelial function as measured by flow-mediated dilation in 40 healthy volunteers. All participants were preloaded with oral methionine to increase plasma homocysteine levels. Intravenous iron reduced endothelial function at 1 h with iron sucrose. At 4 h, endothelial function was similarly reduced in the placebo group compared with the iron group, suggesting that oral methionine load also can impair endothelial function. Although free iron levels were measured, it is unclear whether this was the sole cause of endothelial dysfunction. The study by Rooyakkers et al. (30), described earlier, showed dissociation between free iron and endothelial dysfunction; endothelial dysfunction peaks a lot sooner than the peak in free iron concentration.

**Intravenous Iron Sucrose Causes Acute Renal Injury in CKD**

In a model of obstructive uropathy in mice, Zager et al. (32) showed that intravenous iron generates the proinflammatory chemokine monocyte chemotactrant protein-1 (MCP-1). Whether administration of intravenous iron to people with CKD generates oxidative stress and renal injury was the subject of another study by Agarwal et al. (33). Patients who had CKD and were not on dialysis were given intravenous iron sucrose 100 mg over 5 min. TSAT was measured using urea-PAGE. Plasma and urine samples were collected and assayed for biomarkers of lipid peroxidation (MDA), redox state (plasma and erythrocyte oxidized and reduced gluthionie [oxidized glutathione and reduced glutathione]), antioxidant enzymes, and markers for renal tubular and glomerular injury and/or inflammation (MCP-1, urine total protein, and N-acetyl-β-glucosaminidase). Plasma and urine samples were collected at 0.25, 0.5, 1, 2, 3, and 24 h after intravenous iron sucrose administration. After these studies were performed as baseline for the group of 20 patients, the patients were randomly assigned to receive either N-acetylcysteine (NAC) 600 mg twice a day for 7 d ($n = 10$) or placebo ($n = 10$). Seven days later, another dose of intravenous iron sucrose was administered to all participants, and urine and plasma samples were collected at timed intervals.

The hemoglobin concentrations in the two groups were not changed at 1 wk. In contrast, a single dose of 100 mg of iron increased the ferritin level from 85 to 91 ng/ml for the placebo group and from 127 to 169 ng/ml for the NAC group. These differences were statistically significant. There was a progressive increase in TSAT as measured by urea-PAGE, which did not reach a peak until 3 h after intravenous iron infusion. At approximately 24 h, TSAT reached a level that was approximately twice the baseline level. The peak in TSAT occurred at 3 h after intravenous iron administration. At 15 min, there already was a peak in oxidative stress as measured by plasma MDA. Similar to the findings in normal, healthy volunteers (30), in this study of people with CKD, there was peak generation of oxidative stress within 15 min of intravenous iron infusion. Oxidative stress returned to baseline within 24 h. Similar results were obtained for urinary MDA. Urinary N-acetyl-β-glucosaminidase and protein excretion rates showed a rapid rise and decline to baseline levels in 24 h that paralleled the generation of oxidative stress. In a more detailed analysis of the nature of oxidative stress and injury in these patients, Agarwal (34) showed that acute renal injury and oxidative stress after administration of intravenous iron sucrose produces carbonylation, fragmentation, and loss of immunoreactivity of urinary albumin in a time-dependent manner. Fur-
thermore, consistent with the observations of Zager et al. (32), MCP-1 was generated and excreted rapidly in response to intravenous iron (35). Although oxidative stress was reduced with NAC, it did not affect renal injury and inflammation as measured by proteinuria, enzymuria, and urinary MCP-1 excretion (33,35). Therefore, it seems that intravenous iron sucrose is not only generating free iron and causing oxidative stress and renal damage but also may be causing renal injury directly. This revised model of renal injury with intravenous iron is shown in Figure 4.

Leehey et al. (36) studied iron gluconate, in two different dosages, for its ability to cause oxidative stress and renal injury and also looked for protection from injury with NAC. They performed a four-period crossover study in eight patients with CKD. Sodium ferric gluconate was administered in a dosage of 125 mg over 1 h with or without NAC or a higher dosage of sodium ferric gluconate, 250 mg, over 2 h with or without NAC. Every patient underwent each one of these four therapies. Adult US veterans who had an estimated GFR of 60 ml/min, creatinine levels >1.3 mg/dl, and also looked for protection from injury with NAC. They were compared, likely as a result of the limited power of the study. NAC did not block the oxidative stress response. Despite the generation of oxidative stress, there was no increase in proteinuria or albuminuria even with the higher dosage of iron gluconate. There was an increase in enzymuria, especially at the high dosage, which was not statistically significant probably because of low power of the study.

Conclusion

Intravenous iron generates oxidative stress, inflammation, endothelial dysfunction, and renal injury. The propensity for these events is increased with nondextran iron, but the clinical use of iron dextran, especially the large molecular form, is complicated by anaphylactic reactions and death. There seem to be differences between iron sucrose and iron gluconate in their ability to donate iron directly to transferrin (gluconate > sucrose) and to cause proteinuria and enzymuria (seen with iron sucrose only). Although antioxidants somewhat can mitigate the oxidative stress, they do not limit the renal injury that is caused by intravenous iron sucrose. This is consistent with the hypothesis that intravenous iron may be causing oxidative stress and renal injury by two independent pathways. The short-term experiments described earlier are provocative but not conclusive. The determination of the long-term clinical significance of these observations in patients with CKD or those who are on peritoneal dialysis requires clinical trials.

References


Figure 4. Proposed pathway of injury as envisioned initially (bold arrows) and as modified by the results of the trial (dashed arrows) (33).


